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# Using LEXT laser-scanning confocal microscopy to identify enamel surface defects in archaeological caribou dentition: A pilot study from Southern Baffin Island, Nunavut, Canada



Julia A. Gamble<sup>a,\*</sup>, S. Brooke Milne<sup>b,c,d</sup>

- a Department of Anthropology, Faculty of Arts, University of Toronto, 19 Russell Street, Toronto, Ontario M5S 2S2, Canada
- Department of Anthropology, Faculty of Arts, University of Manitoba, 432 Fletcher Argue Building, 15 Chancellor Circle, Winnipeg, MB R3T 2N2, Canada
- <sup>c</sup> Centre for Earth Observation Science (CEOS), 535 Wallace Building, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
- d ArcTec Research Lab, 303A/B Duff Roblin Building, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

#### ABSTRACT

Dental enamel defects have been used extensively in past human populations to elucidate patterns of health and physiological disruption (often simply referred to as stress). These defects are most commonly assessed through visual examination and used to infer such information as the frequency and age at occurrence of stress events. However, a microscopic approach makes it possible to more consistently identify patterns of growth and growth disruption in greater detail than that possible with traditional macroscopic techniques. Such microscopic studies are being increasingly explored in bioarchaeology, but this area of investigation has not seen extensive application to zooarchaeological material. Consequently, enamel defects in general have not been integrated as heavily in this field. A species of particular importance within the modern context of climate change is the barren-ground caribou (Rangifer tarandus groenlandicus). This species has been a crucial species to the human populations throughout the Arctic for thousands of years, and herd fluctuations have in the past shaped the human experience. The study of past stress in these populations could provide significant historical insight into herd patterns, which would have impacted subsistence and mobility patterns, and traditional use of these animals among both past and contemporary human populations. This paper presents the results of a pilot study using the Olympus LEXT Laser-Scanning Confocal Microscope OLS4000 to evaluate enamel growth in an archaeological caribou tooth from the LdFa-1 site (a large caribou hunting site located in the deep interior of southern Baffin Island). Our study demonstrates the potential of this technology to capture enamel surface microstructures and to provide greater insight into the fine patterns of growth arrest within this zooarchaeological context.

## 1. Introduction

Dental enamel defects act as markers of non-specific systemic stress. As such, they can arise in response to a range of stressors that affect physiological systems during growth and development (Goodman, 1991; Goodman and Armelagos, 1985; Goodman and Rose, 1990; Neiburger, 1990; Pindborg, 1982; Upex and Dobney, 2012). In the case of enamel, ameloblast (enamel forming cell) activity is disrupted, leading to changes in enamel formation (Hillson, 2005, 2014; Witzel et al., 2008). Because enamel does not remodel once formed, this stress marker remains for the rest of the individual's life, unless obliterated through dental attrition.

These markers of physiological disruption on the surface of the tooth are visible as dental enamel hypoplasia (DEH), the most common

of which are linear disruptions known as linear enamel hypoplasia (LEH). When viewed microscopically, LEH can be seen as irregular spacing in the incrementally-forming growth lines (perikymata) on the enamel surface (Bocaege et al., 2010; Bocaege and Hillson, 2016; Elhechmi et al., 2013; Hassett, 2012; Hillson, 1992; Hillson et al., 1999; Hillson, 2014; Hillson and Bond, 1997; Hillson and Jones, 1989; King et al., 2002, 2005; Temple et al., 2012; Witzel et al., 2008). DEH can also manifest as disruptions involving single or multiple perikymata (furrow-form defects), as broader patches of disruption involving extended exposure of a single growth line (also known as plane-form defects) or as discontinuous patches (pit-form defects) (Hillson, 1996, 2014). Internally, growth disruption is manifest as accentuated striae of Retzius (AS), with striae of Retzius being the internal incremental

<sup>\*</sup> Corresponding author at: Department of Anthropology, University of Toronto, 19 Russell Street, Room 340, Toronto, ON M5S 2S2, Canada. E-mail addresses: Julia.gamble@utoronto.ca (J.A. Gamble), Brooke.Milne@umanitoba.ca (S.B. Milne).

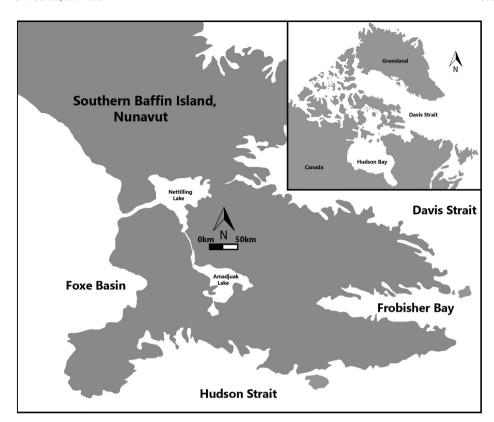


Fig. 1. Map showing the locations of Nettilling and Amadjuak lakes on Baffin Island in relation to the study site (LdFa-1).

(modified from original in Milne, 2003)

structures visible on the surface a perikymata (Goodman and Rose, 1990; Hillson, 1996, 2014; Witzel et al., 2008). LEH in particular have formed an important component of bioarchaeological research into patterns of stress in past human populations and are most commonly interpreted as evidence for nutritional deficiency or infectious disease (El-Najjar et al., 1978; Goodman et al., 1980; Goodman, 1996; Goodman and Rose, 1990). However, the non-specific nature of these markers should be taken as a cautionary point against over-simplification of etiological inferences (Neiburger, 1990).

While DEH has the potential to provide insight into patterns of physiological disruption during development in non-human mammals, the study of enamel defects in zooarchaeological research is limited with the exception of a few notable examples that focused on suids (Dobney et al., 2002, 2004; Dobney and Ervynck, 1998, 2000; Ervynck and Dobney, 1999; Upex et al., 2014; Wang et al., 2012) and bovids (Byerly, 2007; Kierdorf et al., 2006; Niven, 2000; Niven et al., 2004); paleontological studies have also been conducted on a few fossil species (Franz-Odendaal et al., 2003; Mead, 1999). More varied studies have been done on a range of living mammals (Franz-Odendaal, 2004; Kierdorf et al., 1991, 2006, 2012, 2016; Mellanby, 1929, 1930; Suckling, 1980; Suckling et al., 1983, 1986; Suckling and Purdell-Lewis, 1982; Upex and Dobney, 2012). A species of particular interest for the study of human adaptation in the Arctic is the caribou (Rangifer tarandus groenlandicus). Caribou have been an essential subsistence and material resource for circumpolar human populations for millennia given their invaluable hides for warm clothing; meat and marrow for food; and their bones, antler, and sinew for construction materials (Stenton, 1989, 1991a, 1991b, 2001). Caribou are vulnerable to dramatic cyclical fluctuations in population stability, which can last years to decades (Milne and Donnelly, 2004; Stenton, 1991a). Causes for herd instability are frequently attributed to climatic shifts, predation, disease, starvation, and over-hunting (e.g. Burch, 1972; Darwent, 2004; Gordon, 1974; Meldgaard, 1983; Messier et al., 1988).

Conceivably, the stress caused by these factors should be detectable as DEH on caribou teeth, and the ability to track such stress indicators

could provide insight into patterns in herd fluctuation both in the past and in the present. Preliminary work has successfully identified LEH in caribou molars from modern herds in the Northwest Territories (Wu et al., 2012). This seminal study developed age at formation estimates for the first, second, and third molars using radiographs. With this information, in addition to photographs taken at up to  $25 \times$  magnification, Wu et al. (2012) successfully identified and assessed the presence of LEH in their study population.

Extensive studies of human teeth demonstrate the importance of examining enamel defects microscopically (Hassett, 2011, 2012; Hillson, 2014). Traditionally, this has been accomplished using scanning electron microscopy (SEM), or an engineer's measuring microscope (Hillson, 2014; Hillson and Bond, 1997; Hillson and Jones, 1989; King et al., 2002, 2005). More recent technologies, including differential focus variation (Bocaege et al., 2010) and laser-scanning confocal microscopy (Gamble, 2014; Gamble and Milne, 2016), have been used to capture enamel surface topographies. Such technology has not previously been applied to caribou teeth, either in modern or archaeological populations, despite the demonstrated utility of microscopic level investigations in human teeth.

This paper presents the results of a pilot study that applies for the first time the Olympus LEXT laser-scanning confocal microscope OLS4000 to examine the dental enamel surface topography of zooarchaeological samples of caribou teeth on a microscopic level. Our aim is to assess if the LEXT can accurately capture profile lines that can be used to objectively identify and quantify enamel defects along the crown surface in caribou dentition. We acknowledge the sample size for our study is exceedingly small (i.e. N=1); however, our principal objective in this pilot study is to demonstrate proof of concept so that our methods can be refined as needed to facilitate future applications on notably larger zooarchaeological samples. Thereafter, more robust data collection can proceed, yielding quantifiable results that will provide insights on caribou herd dynamics over time.

Our study sample derives from the LdFa-1 site, which is located in the deep interior of southern Baffin Island. LdFa-1 is strategically

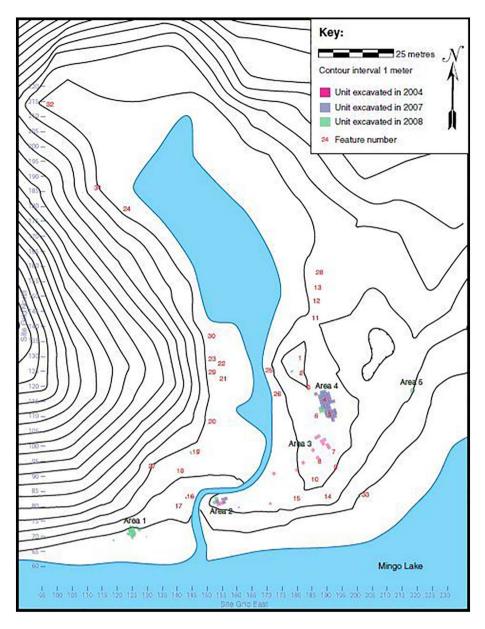


Fig. 2. Topographic map showing the LdFa-1 site with the location of the five excavation areas, with the tooth used in this study found in Area 4.

(modified from original in Park (2009) and reprinted from Thacher (2017: Fig. 2) with permission from Elsevier)

situated to take advantage of large resident caribou herds that migrate through the region (see Fig. 1). Archaeological investigation indicates site occupants were principally focused on hunting and processing these animals over a 3500-year period (McAvoy, 2014; Milne et al., 2012, 2013). Our preliminary results establish a method for examining non-specific stress patterns in this vitally important Arctic terrestrial species, and its future applications on larger zooarchaeological samples will provide crucial insights on how caribou population cycles may have impacted human subsistence and settlement in this region over time. Moreover, contemporary applications have the potential to inform how ongoing herd dynamics may be linked and/or exacerbated by Arctic climate change (e.g. Jenkins et al., 2012).

# 2. Materials

# 2.1. Study area

The interior landscape of southern Baffin Island is dominated by two of the largest lakes in the Canadian Arctic Archipelago – Nettilling and Amadjuak (Fig. 1). These lakes and the tributary rivers and drainage basins that connect them create a warmer, more stable climate

compared to other regions throughout the summer, fall, and early winter months (Jacobs and Grondin, 1988, p. 212). The island's interior supports a rich and reliable subsistence resource base that includes an abundance of plants, fresh water fish, nesting waterfowl, foxes, wolves, and caribou (Boas, 1964; Milne and Donnelly, 2004; Soper, 1928; Stenton, 1989). Polar bears are even known to travel inland albeit infrequently (Milne, 2005a). Another essential material resource, particularly for the earliest human inhabitants, is chert toolstone. Chert is found localized in the interior and the intermediate zone separating it from southern Baffin Island's coasts (Milne, 2005b; ten Bruggencate et al., 2015, 2016a, 2016b, 2014). This warmer climate and reliable resource base made southern Baffin Island's interior extremely attractive for human occupation, and archaeological evidence attests to its repeated, intensive long-term use for millennia (Milne, 2003, 2005b; Milne et al., 2012, 2013; Stenton, 1989, 1991a, 1991b, 1991c).

Caribou have been an essential source of food, hides, and building materials for all Arctic human populations (Burch, 1972, p. 339). Baffin Island supports several large resident herds (Maxwell, 1985, p. 82), the largest of which is the south Baffin herd with estimated numbers from tagging projects conducted in the 1980's exceeding 60,000 animals (Stenton, 1989, p. 96). Caribou are available year round on Baffin Island

**Table 1**Technical specifications for Olympus LEXT laser-scanning confocal microscope OLS4000 objective lenses used in this study.

Lens name	Lateral magnification	Total magnification	Numerical aperture	Working distance (mm)	Field of view (mm)
MPlanFLN2.5x	2.5 ×	25 ×	0.08	10.7	8.8
MP LFLN10x	10 ×	216 ×	0.30	11.0	12.8
MP LAPON20xLEXT	20 ×	432 ×	0.60	1.0	6.4
MP Lapon50xLEXT	50 ×	1,080 ×	0.95	0.35	2.56

but their distributions vary (Soper, 1928). Most caribou populations make two definite seasonal movements – to a calving area in the spring and summer, and to a winter range in the fall (Burch, 1972, p. 345). While migratory routes between these destinations can and do vary, the location of calving grounds tends not to change thus making them reliable locations for hunters to find caribou (Gordon, 1996, p. 12).

Despite this seeming abundance of caribou on southern Baffin Island, these herds, like all others, experience dramatic cyclical fluctuations (e.g. Burch, 1972; Gordon, 1974; Meldgaard, 1986; Messier et al., 1988; Stenton, 1991a). As Meldgaard (1986) notes for herds in Western Greenland, these declines may last between 35 and 70 years; thus, creating sporadic and sharp declines in local numbers, if not a complete disappearance. When the south Baffin herds decline, the remaining animals tend to contract to the inland region and are, therefore, unavailable along the coast (Stenton, 1989, 1991a, 1991b). This forced human populations to travel long distances inland during these times to find caribou (Stenton, 1991a, p. 28). A more recent survey of Baffin Island's caribou herds indicates that within the last 20 years, total herd numbers have fallen from estimated highs of 180,000 animals to between 1065 and 2067; a decline of 95% (Jenkins et al., 2012, pp. i-ii). While the causes for this dramatic crash are not yet entirely understood, Inuit hunters who still rely on caribou as an important subsistence and material resource acutely feel its impact.

#### 2.2. LdFa-1

LdFa-1 is an expansive archaeological site strategically situated near a naturally occurring narrow on the northwest shore of Mingo Lake on Baffin Island. This narrow sees significant caribou traffic where large numbers of animals habitually cross from the lake's southern shore to the north. When they exit the water, the caribou immediately face the Mingo Lake esker—a steep glacial feature extending several kilometers along the lake's northern margin. As the animals exit the water they are virtually blind to what lies above them, thereby providing hunters with an ideal situation to dispatch them as they ascend the esker to move overland down its other side. There are at least three large hunting blinds situated within 100–150 m from LdFa-1 where hunters undoubtedly waited for the animals to pass by.

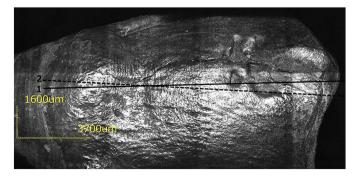


Fig. 3. Tooth crown showing positioning of profile paths, with the path numbered "1" representing the average path used for the first three paths and the path numbered "2" representing the path used for the fourth path. As can be seen in this image, the perikyma orientation is slightly variable across the crown, with this shift being apparent in particular on opposite sides of the main defect. The fourth path was established on a different angle to test whether this would change the results.

Archaeological investigation of LdFa-1 has revealed occupations dating to the Pre-Dorset (4000–2500 BP), Dorset (2500–1100 BP), and Thule Inuit (1100 BP – present) periods. The level of organic preservation at the site is remarkable. High frequencies of delicate organic artifacts, including sewing needles, suggest site occupants were actively working caribou hides into clothing and other items. Bone debitage indicates organic tools were also being made. Most striking, however, is the massive faunal assemblage, which for the Pre-Dorset component alone exceeds 16,000 specimens that are 98% caribou (McAvoy, 2014; Milne et al., 2012, 2013).

#### 3. Methods

This pilot study involved the imaging of the buccal view of a single mandibular third molar excavated from a Pre-Dorset era deposit known as Feature 4 in Area 4 of the larger LdFa-1 site (see Fig. 2). Feature 4 consists of a well-defined ring of stones that demarcate the location of an ancient skin tent dwelling that was occupied during the Arctic summer months by the Pre-Dorset peoples (McAvoy, 2014; Thacher et al., 2017). Heavy rocks like these were used to anchor the edges of skin tents to the ground so that the structures could withstand strong Arctic summer winds (Milne and Park, 2016).

The tooth was cleaned thoroughly using dry brushing, followed by the combination use of cotton swabs and a toothbrush with ethanol. It was observed and photographed before imaging, with enamel defects being identified macroscopically at this level. The full tooth crown was then imaged using the Olympus LEXT laser-scanning confocal microscope OLS4000 using the  $2.5\times$  MPlanFLN lens (25  $\times$  magnification) to capture the full crown. The  $2.5\times$  lens image capture was done with polarized illumination as per the lens specifications (http://www.olympus-ims.com/en/metrology/ols4000/). This was followed by imaging with the  $10\times$  (MPlanFLN10  $\times$ ) and  $20\times$  (MPlanApoN20  $\times$ ) lenses for profile line extraction, and further image capture with the  $50\times$  lens to gain better insight into the finer enamel microstructures in the form of Tomes process pits (Table 1).

Profile lines were extracted from both the  $10\times$  and  $20\times$  images using the Olympus LEXT OLS4100 Desktop application (version 3.9.1.2) and examined further in GraphPad Prism 7.03. Placement of the first three profile lines was established on the mesial crown and positioned to capture a longitudinal section along the midline, with the aim of running directly across (perpendicular to) the perikymata (Fig. 3). These three lines were all established at independent times, but with the aim of testing replicability of positioning (i.e. they were positioned to capture the same path). The fourth path was placed at a more oblique angle across the section as perikymata for the caribou molar were irregular in their spacing and angles across the crown. This last path was therefore designed to test whether a different angle would capture the same patterns (Fig. 3). There were no appreciable differences in surface topography between the different paths either at the  $10\times$  or  $20\times$  levels, and so the first path was used for all analyses (Fig. 4).

Further analysis of the LEXT images was conducted using GraphPad Prism 7.03. Following the work of Hassett (2012, 2014) and the recent work of Cares Henriquez and Oxenham (2017) teeth were evaluated for perikymata spacing, and, similar to Cares Henriquez and Oxenham (2017), for surface divergence in contour. Hassett (2014, 2012) used

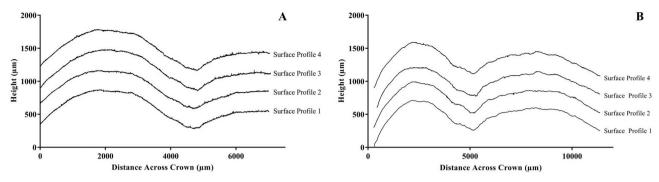
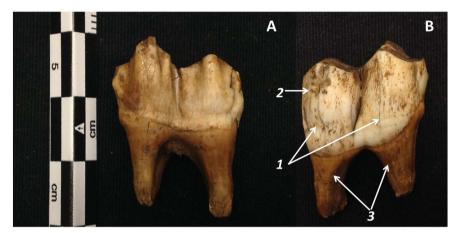


Fig. 4. Path comparison for profile lines extracted from the  $10 \times (A)$  and  $20 \times (B)$  images.



**Fig. 5.** Lingual (A) and buccal (B) views of sample #773 showing a linear defect near the cementum-enamel junction (1) and pit-form defect higher on the crown (2). Accentuated rings are also visible on the root of this tooth (3).

the moving average of perikymata spacing to identify divergence from the normal spacing by crown region. Cares Henriquez and Oxenham (2017) applied this approach, but also developed the micro polynomial method in which a 6th order polynomial was used to calculate the residual in relation to moving average before testing this in relation to the degree of standard deviation. This latter method is proposed as a compensatory method where continuous perikymata are not visible in relation to crown surface (Cares Henriquez and Oxenham, 2017), and given low visibility of perikymata in the caribou tooth examined here, due to a combination of morphology and wear, such a mixed approach had potential value.

In the current study, the main curve was extracted using the Olympus LEXT OLS4100 software and manually fit with a multi-point spline set to the high points along the length of the crown. This approach was taken as it presented the opportunity to produce a better fitting curve than the higher order polynomial approaches. Perikymata were marked visually as point registrations with Olympus Stream Desktop (version 2.2) and the coordinates were exported for analysis in GraphPad 7.03, which made it possible to easily evaluate fit of polynomial regressions. Intraobserver error in perikymata identification was also evaluated with five different trials run over the course of this project at each level of magnification (i.e. using the  $10 \times$  and the  $20 \times$ lenses). The first trial was offset from the second to fifth trials by a period of four months. In response to irregular identification of perikyma across the trials, only perikyma that were identified consistently three or more times out of the five trials were used for analysis. For inclusion, the perikyma had to be positioned within 28.8 µm of a perikyma identified in another trial, the equivalent of half the minimum perikymata spacing across the imaged portion of the crown (57.61 μm).

# 4. Results and discussion

# 4.1. Visual and low magnification assessment

Visual (macroscopic) inspection of the tooth after cleaning revealed clear linear defects on the crown, with the most significant one being on the mesial portion of the crown (protoconid) and clearly visible on the buccal surface. This same defect was also apparent in the distal portion of the crown (hypoconid) as two separate LEH (Fig. 5). It is also notable that both periradicular bands and an accentuated ring are visible on the root of this tooth, representing what has been proposed as equivalent to striae of Retzius and accentuated striae of Retzius in internal enamel (Dean, 1995; Kawasaki et al., 1979; Smith, 2008; Smith and Reid, 2009) (Fig. 5).

The  $2.5 \times$  lens, representing  $25 \times$  magnification, is the highest magnification possible for full crown capture with the LEXT stitching function. While the LEXT software should have the capacity for additional stitching after image capture, further work is needed to optimize this for full crown capture. Attempts at stitching after image capture have thus far resulted in stitching of images vertically as well as horizontally, even when path instructions stipulated only a horizontal stitching pattern. Initial testing was therefore unable to deal with full crown capture using this software. At  $25 \times$  magnification, perikymata are visible (Fig. 6) and the profile line clearly identifies crown relief with both the linear and pitform hypoplastic episodes. At this magnification, it is therefore possible to easily capture the larger defects with a profile line but not to objectively identify perikymata or perikymata spacing for the identification of smaller enamel growth disruption, as the numerical aperture of 0.08 (2.5  $\mu$ m resolution) is insufficient for perikymata capture.

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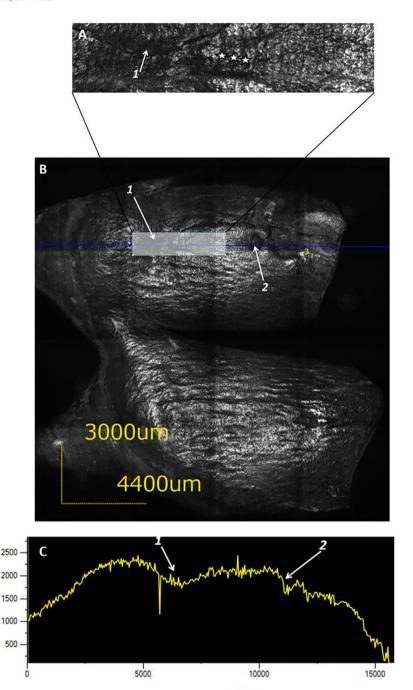


Fig. 6. Tooth crown captured with the  $2.5 \times$  lens with A) representing the digital zoom showing visible perikymata marked by stars, (1) showing the linear defect in the profile line, full image, and digital zoom, and (2) showing a pit-form defect on both the full image and profile line.

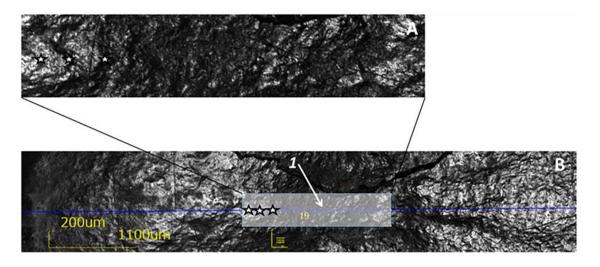
Imaging with the  $10 \times$  lens results in a far smoother profile line due to the higher resolution (0.68 µm as opposed to 2.5 µm with the  $2.5 \times$  lens) (Fig. 7). While some of the more pronounced perikymata are captured, they are not distinguished sufficiently to identify them through the z-plane. Instead, they were identified and marked visually on the image and measurement data on spacing was generated (Fig. 8).

Perikyma spacing showed the most significant fit with a 2nd order (quadratic) polynomial regression at this magnification. Consideration of perikymata spacing showed nine points along the crown, which also appeared as depressions in the crown surface in relation to the fitted spline curve (Fig. 8). Disruption in perikyma spacing is visible towards the occlusal edge of a number of these defects; specifically, they are visible as increased spacing between perikymata. However, the conservative inclusion of perikymata in the composite line means that the line may not

fully capture all perikymata. Indeed, the intraobserver testing showed relatively low repeatability, with a maximum of 51 perikymata being identified in the first trial (compared to 41, 43, 41, and 39 perikymata in trials two to five, respectively). Of these, 35 were identified in a minimum of three trials. The distances generated therefore reflect replicability of 68.6% to 81.4%, and so the plotted perikymata spacing may fail to capture some of the nuances of spacing in relation to crown surface topography. Nevertheless, the most significant defects do correspond to increased perikymata spacing, even at this level of magnification.

#### 4.2. Higher magnification identification of perikymata

The 20  $\times$  lens resulted in far higher resolution (0.34  $\mu$ m compared to 0.6  $\mu$ m seen with the 10  $\times$  lens), allowing more consistent capture of



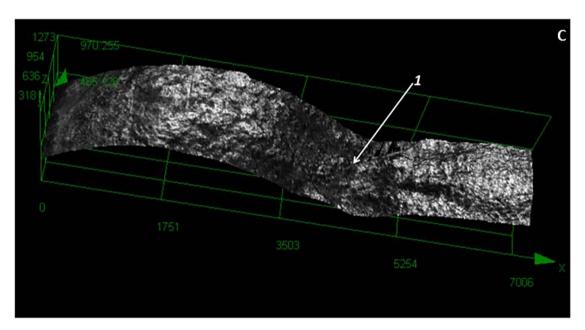


Fig. 7. Tooth crown captured with the  $10 \times lens$  with A) representing the digital zoom showing visible perikyma marked by stars and (1) showing the linear defect in the profile line, full image, and digital zoom.

perikymata in relation to noise. This is most clearly illustrated in Fig. 9, where it is possible to look closer at the linear defect identified at lower magnifications and to gain further insight into the pattern of growth arrest experienced by this individual.

The extracted profile was plotted next to a spline-adjusted curve applied in the Olympus LEXT OLS4100 software. Perikymata were more visible in profile than visually at this level, and it is notable that in a number of areas (particularly along the highest curve of the crown) they were noticeably difficult to identify, leading to inconsistent visual scoring for evaluation of perikymata spacing. This is reflected in a high rate of variability in the total number of perikymata identified across the five trials, with 60, 78, 83, 66, and 51 perikymata identified in each respective trial. A total of 28 perikymata (33.7%–54.9%) were identified in three or more trials. The particularly low repeatability through visual evaluation here may reflect the narrower longitudinal section of the crown being captured with less ability to view the broader perspective of perikymata. It may also reflect a combination of the portion of the crown captured, with the high level of differential expression and

orientation of visible perikymata being clear even with the  $10\times$  lens. However, the same portion of the crown was captured with the  $10\times$  lens as with the  $20\times$  lens, and other areas within this capture region produced similar low visibility of perikymata. This suggests that the narrowness of the longitudinal band may be reducing visual cues.

It is in these instances that the comparison of the surface curvature in relation to the spline curve, which showed the best fit to the tooth surface curve, can be most useful in identifying defects. This method is similar to that applied by Cares Henriquez and Oxenham (2017) in that it compensates for perikymata that cannot be scored through the consideration of surface topography. A core difference is the use of the spline-adjusted curve making use of the Olympus LEXT OLS4100 fitting function rather than a fixed higher order polynomial (Cares and Oxenham, 2017 used a 6th order polynomial for their analysis). At the  $20 \times$  level, the limitations in perikymata visibility led to the identification of ten instances where the curve divergence and perikymata spacing both identified the same defect (Fig. 10). Perikymata are also particularly visible on the profile line in these instances. Defects

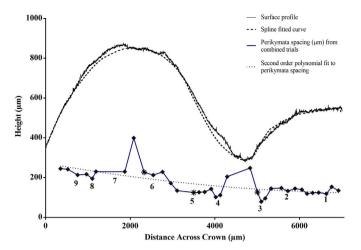


Fig. 8. Extracted profile of crown 772 taken at  $10 \times magnification$  and shown in relation to the spline fitted curve and the perikymata spacing across the crown. This shows nine points of irregular perikyma spacing which are also reflected by divergence of the crown from the spline curve, with the most prominent being those for defect 1 (associated with numbers 3 and 4 in this figure), and those involved in the large defect marked by points 6 and 7. Asterisks indicate examples of perikyma leading into periods of disrupted development, marked by longer spacing of perikymata.

numbered 4 through 8 represent the largest defect in the crown, with multiple episodes of disruption (marked by increased spacing of perikymata) and recovery involved (also visible in Fig. 5).

When considering Figs. 8 and 10, the crown develops from the cusp to the root, and so it begins development from the right side of the images (with the lower-numbered defects) and then progresses to the left towards the higher-numbered defects. This means that the lowest point, marked by a perikyma within the enamel defect (number 7), and marked by "1" in Fig. 9, is preceded by the disrupted growth episode beginning to the right, at the 'normal' level of the crown surface (indicated by the asterisk in both figures). In this individual, recovery

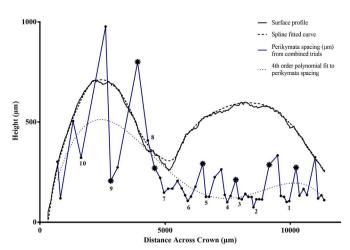


Fig. 10. Extracted profile of crown 772 taken at  $20 \times$  magnification and shown in relation to the spline fitted curve and the perikymata spacing across the crown. Consideration of these curves shows seven points of irregular perikymata spacing which are also reflected by divergence of the crown from the spline curve. This comparison in particular captured abnormally spaced perikymata moving into the defect associated with numbers 6 to 8 and 3. Asterisks indicate examples of perikyma leading into periods of disrupted development, marked by longer spacing of perikymata.

began after this point, and as we proceed to the left, the height of the profile increases. This most significant disruption marked by numbers 4 to 8 occurs later in crown development and is visible at lower magnifications. However, in viewing the profile lines generated with the  $20 \times 10^{10}$  lens (Fig. 10), it is clear that this defect can be divided into two periods of disruption, with the second occurring during recovery from the initial episode (marked as defect number 8 in Fig. 10). The disruption here begins with the farthest right perikyma marked by the arrows in Fig. 9a. This parallels the second hypoplastic episode visible on the distal portion of the molar crown.

Further histological work on enamel formation schedules for



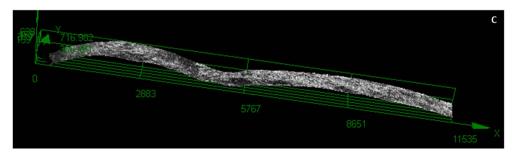


Fig. 9. Tooth crown captured with the  $20 \times lens$  with A) representing the digital zoom showing visible perikymata marked by stars, B) showing the full longitudinal image of the crown, and C) showing the three-dimensional rendering of the section of crown captured at this magnification.



Fig. 11. Crown surface captured with the  $50 \times$  lens showing the honeycombed effect of Tomes process pits.

caribou would provide additional insight into the timing and relationship of such defects. The surface visibility of perikymata is also clearly irregular, suggesting the utility of a combination approach to defect identification. Work on other mammals with high-crowned molars has suggested interference of factors such as coronal cementum and enamel extension rate (Kierdorf et al., 2006, 2012), although this has not been evaluated histologically in caribou teeth. While the current results have suggested the presence of multiple consecutive linear enamel defects, it is possible that if caribou molars follow a similar pattern to other species (i.e. sheep, goat), the current external investigation may have missed other forms of defect such as plane-form defects, particularly in the more cervical portion of the capture range (Kierdorf et al., 2006, 2012). Cementum was not apparent though on visual inspection, and identification of Tomes process pits with the  $50 \times$  lens (Fig. 11) along with some clear perikymata identified visually and through the extracted profile line at lower magnifications, suggests that in this case any coronal cementum will be thin and that the enamel defects identified are, indeed, enamel defects. The association of multiple defects as representing more than one episode of enamel disruption would also require further histological investigation to evaluate whether a similar high enamel apposition rate to that observed in other species could lead to multiple surface occurrences along the crown being linked to a single (rather than multiple) episodes (Kierdorf et al., 2012, 2013).

#### 5. Conclusion

Barren ground caribou have been a key species to human subsistence on southern Baffin Island since the Pre-Dorset period (4000–2500 BP). However, an alarming and precipitous drop of 95% among the island's contemporary resident herds (Jenkins et al., 2012, pp. i–ii) underscores the urgency to identify and understand sources of stress and their impact among these animals. Archaeology as a field of study is uniquely positioned to contribute to these efforts by offering long-term insights on herd population cycles, and possible human impacts including over hunting (e.g. Darwent, 2004). By examining these phenomena diachronically, including the identification of specific stressors and their duration, including those that may affect certain groups within the herds (i.e. cows and calves), it may be possible to predict patterns of herd recovery and stabilization. Such results will be crucial for informing future conservation efforts on southern Baffin Island presently, and the Arctic more broadly.

The LdFa-1 site has produced the largest and best-preserved terrestrial faunal assemblage dating to the Pre-Dorset period on southern Baffin Island (McAvoy, 2014; Milne et al., 2012, 2013). The site's strategic location on the northwest shore of Mingo Lake and proximity to calving grounds for the resident southern Baffin herds provides an ideal opportunity to explore the long term human exploitation of caribou in this region. Moreover, the predominance of well-preserved caribou bones, including teeth, will through future studies, allow us to better understand the overall health and stability of the herds from a broad temporal perspective. While bioarchaeological studies of enamel defects in humans are now well-established, only one study has considered enamel defects in caribou, most specifically among extant populations in the western Arctic (Wu et al., 2012). The study of human enamel defects has also emphasized the importance of microscopic approaches to enamel defect analysis, as such a level of analysis can capture more detail with regards to stress episodes (Bocaege et al., 2010; Cares Henriquez and Oxenham, 2017; Hassett, 2011, 2014; Hillson, 2014).

This pilot study using the Olympus LEXT laser-scanning confocal microscope OLS4000 for the first time on archaeological caribou teeth has led to the successful identification of enamel defects on a caribou  $M_1$ . Furthermore, through the use of a 20  $\times$  lens, representing 432  $\times$ magnification, surface markers of growth (known as perikymata) were identified in association with divergence in normal surface curvature. The utility of this level of analysis is further borne out by its success in identifying a second defect that was otherwise not apparent to the naked eye within a larger linear defect. Both defects are more clearly delineated visually on the distal portion of the crown, but were not visible otherwise on the mesial portion. This highlights the potential for laser-scanning confocal microscopy to provide more detailed insight into patterns of stress in archaeological populations of caribou, thus adding a deep time dimension against which to consider contemporary conservation efforts. Future histological and microscopic work, both on archaeological and modern caribou, will be fundamental to establishing a more accurate framework for developmental schedules and patterns in defect formation. Such work is critical to further develop the methodological framework and to establish a baseline for consideration of the archaeological herd structures.

#### Conflicts of interest

The authors declare that they have no conflict of interest.

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